## Differentiation of RPE cells from integration-free iPS cells and their cell biological characterization.

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**Public Summary:**
Dysfunction of the retinal pigment epithelium (RPE) is implicated in numerous forms of retinal degeneration. The readily accessible environment of the eye makes it particularly suitable for the transplantation of RPE cells, which can now be derived from autologous induced pluripotent stem cells (iPSCs), to treat retinal degeneration. For RPE transplantation to become feasible in the clinic, patient-specific somatic cells should be reprogrammed to iPSCs without the introduction of reprogramming genes into the genome of the host cell, and then subsequently differentiated into RPE cells that are well characterized for safety and functionality prior to transplantation. METHODS: We have reprogrammed human dermal fibroblasts to iPSCs using nonintegrating RNA, and differentiated the iPSCs toward an RPE fate (iPSC-RPE), under Good Manufacturing Practice (GMP)-compatible conditions. RESULTS: Using highly sensitive assays for cell polarity, structure, organelle trafficking, and function, we found that iPSC-RPE cells in culture exhibited key characteristics of native RPE. Importantly, we demonstrate for the first time with any stem cell-derived RPE cell that live cells are able to support dynamic organelle transport. This highly sensitive test is critical for RPE cells intended for transplantation, since defects in intracellular motility have been shown to promote RPE pathogenesis akin to that found in macular degeneration. To test their capabilities for in-vivo transplantation, we injected the iPSC-RPE cells into the subretinal space of a mouse model of retinal degeneration, and demonstrated that the transplanted cells are capable of rescuing lost RPE function. CONCLUSIONS: This report documents the successful generation, under GMP-compatible conditions, of human iPSC-RPE cells that possess specific characteristics of healthy RPE. The report adds to a growing literature on the utility of human iPSC-RPE cells for cell culture investigations on pathogenicity and for therapeutic transplantation, by corroborating findings of others, and providing important new information on essential RPE cell biological properties.

**Scientific Abstract:**
BACKGROUND: Dysfunction of the retinal pigment epithelium (RPE) is implicated in numerous forms of retinal degeneration. The readily accessible environment of the eye makes it particularly suitable for the transplantation of RPE cells, which can now be derived from autologous induced pluripotent stem cells (iPSCs), to treat retinal degeneration. For RPE transplantation to become feasible in the clinic, patient-specific somatic cells should be reprogrammed to iPSCs without the introduction of reprogramming genes into the genome of the host cell, and then subsequently differentiated into RPE cells that are well characterized for safety and functionality prior to transplantation. METHODS: We have reprogrammed human dermal fibroblasts to iPSCs using nonintegrating RNA, and differentiated the iPSCs toward an RPE fate (iPSC-RPE), under Good Manufacturing Practice (GMP)-compatible conditions. RESULTS: Using highly sensitive assays for cell polarity, structure, organelle trafficking, and function, we found that iPSC-RPE cells in culture exhibited key characteristics of native RPE. Importantly, we demonstrate for the first time with any stem cell-derived RPE cell that live cells are able to support dynamic organelle transport. This highly sensitive test is critical for RPE cells intended for transplantation, since defects in intracellular motility have been shown to promote RPE pathogenesis akin to that found in macular degeneration. To test their capabilities for in-vivo transplantation, we injected the iPSC-RPE cells into the subretinal space of a mouse model of retinal degeneration, and demonstrated that the transplanted cells are capable of rescuing lost RPE function. CONCLUSIONS: This report documents the successful generation, under GMP-compatible conditions, of human iPSC-RPE cells that possess specific characteristics of healthy RPE. The report adds to a growing literature on the utility of human iPSC-RPE cells for cell culture investigations on pathogenicity and for therapeutic transplantation, by corroborating findings of others, and providing important new information on essential RPE cell biological properties.